D2 SEM

AU9517793

(12) PATENT ABSTRACT (11) Document No. AU-A-17793/95
(19) AUSTRALIAN PATENT OFFICE

(54) Title
FIBRE MEASUREMENT

(51)[6] International Patent Classification(s)
G01N 001/28

(21) Application No. : 17793/95 (22) Application Date : 01 05 95

(30) Priority Data

(31) Number	(32) Date	(33) Country
PM5783	23.05.94	AU AUSTRALIA

(43) Publication Date : 30.11.95

(71) Applicant(s)
COMMONWEALTH SCIENTIFIC & INDUSTRIAL RESEARCH ORGANISATION

(72) Inventor(s)
NAME NOT GIVEN

(74) Attorney or Agent
GRIFFITH HACK & CO , GPO Box 1285K, MELBOURNE VIC 3001

(57)

A method of characterising fibre follicle populations in an animal is disclosed which comprises the steps of cropping fibre from a selected area of the skin of the animal and applying to the selected area a suitable material such as vinyl, polysiloxane resins which are adapted to conform closely to and thereby form a detailed impression of the skin surface. The resins may be heated to place them in a fluid condition at the time of application. After setting the set resin is removed to provide a formed impression and the formed impression is examined to determine the incidence of solitary fibres, bundles of fibres and the number of fibres per bundle

AUSTRALIA

Patents Act 1990

COMPLETE SPECIFICATION

STANDARD PATENT

Applicant(s):

COMMONWEALTH SCIENTIFIC & INDUSTRIAL RESEARCH ORGANISATION

Invention Title:

FIBRE MEASUREMENT

The following statement is a full description of this invention, including the best method of performing it known to me/us:

This invention relates to methods for investigating the nature and distribution of fibres growing from animal skin.

Traditionally, goods made from wool for the mass market have been based on wool having an average fibre diameter of about 23μ. It is, however, recognised that the appearance and feel of wool products improves as greater use is made of fibres having an average diameter less than 23μ, and recent years have seen more extensive use being made of the finer fibres in a bid to enhance the market appeal of wool fabrics. This in turn has lead to an increasing interest in selecting sheep for fine wool growth, and in methods which facilitate such selection.

Figure 1 is a longitudinal section of sheep skin. Typically, wool grows as solitary fibres produced in primary (P) or unbranched original secondary (SOU) follicles, each of which emerges from the skin spaced apart from the other, or it grows in the form of bundles of fibres comprising a fibre produced in a branched original secondary (SOB) follicle together with one or more fibres produced in derived secondary (SD) follicles branching from the SOB follicle. SD follicles attach to each other and to the SOB follicle near the skin surface so that they frequently share a common opening to the skin surface or emerge from the skin surface in close association with other SD follicles. Measurement of the density of the various types of fibres, the density of fibre

bundles and the number of fibres per bundle provides a basis for estimating the density of follicles which initiate from the epidermis (N_E), the density of SD follicles (N_{SD}), and the number of SD follicles per follicle bundle (n_B). Current understanding of the biochemical mechanisms controlling follicle initiation and formation of the fibre in the bulbs of mature follicles indicates the likelihood of a strongly positive genetic correlation of N_{sc} and n_b with clean fleece weight, and a strongly or significantly negative genetic correlation of N_z, N_{sc} and n_b with average fibre diameter. Accordingly, selecting stock for increases in N_E, N_{sc} and n_b can be expected to lead to increases in clean fleece weight and reductions in fibre diameter.

Conventional procedures for studying wool follicle populations have involved inspection of transverse or longitudinal sections of an animal's skin. To identify bundled follicles and to obtain reliable estimates of the density of the follicle bundles and the number of follicles per bundle, requires the preparation of numerous serial sections of the skin. In addition to being invasive, the procedure is difficult, tedious and expensive, and consequently cannot be expected to find widespread application in the breeding industry; even as a tool in breeding research, serial sectioning is only rarely used.

The present invention makes it possible to measure and study follicle populations at relatively low cost and without recourse to sectioning. In one aspect it offers a method of characterising fibre follicle populations in an animal which comprises the steps of:

(a) cropping fibre from a selected area of the skin;

(b) applying to the selected area a settable material adapted to conform closely to and thereby form a detailed impression of the skin surface;

(c) removing the so-formed impression and examining it to determine the incidence of solitary fibres, bundles of fibres and the number of fibres per bundle.

A further aspect of the invention is the use of fibre population characteristics determined by the above method as a basis for selecting sheep for fine wool production.

In this specification, by "cropping" we mean reducing the length of emerging fibres sufficiently to substantially prevent them bending when a settable material is applied. Bent fibres are undesirable as they can give rise to an impression on which important details are distorted or obscured by fibres extending along, rather than away from the skin surface; in

the case of sheep, the problem will usually be avoided if fibres are cropped to a length of less than about 0.5mm. It is expected that most operators would elect to shorten the fibres by mechanical means, e.g. with a razor, and while chemical cropping is not ruled out, clearly the use of chemicals introduces a risk of reactions which might modify fibre follicles and other skin features.

By "settable material" we mean a material which will penetrate the interstices and conform to the contours of the skin without significantly distorting the surface features; it should set rapidly to form a rigid, or semi-rigid, faithful impression of the skin surface features, and be readily removable from the skin after setting. By way of example, we have found vinyl polysiloxanes (commonly the basis of dental resins) to be very satisfactory for the purposes of this invention.

Examination of the skin impression may be carried out by optical or other magnifying means. Preferably it is carried out by scanning electron microscope (SEM). An unexpected and particularly attractive feature of this invention is the ease with which it is possible to recognise and discriminate between fibre and other skin surface features on scanning the impression by electron microscope. A fibre, whether solitary or in a bundle, appears as a dark, more or less circular hole surrounded by an annulus which is light in shade relative to

surrounding regions of the skin. This makes fibres readily distinguishable from other features in the impression, including small defects, and should greatly facilitate the adoption of computerised image analysis techniques for the measurement of the density and relative location of fibres in a skin sample. Furthermore, fibres in bundles can be distinguished from solitary fibres without difficulty since they are located much more closely to each other than are solitary fibres; also their annuli frequently merge together so that on inspection by SEM they appear as a number of circular holes grouped together within a region which appears light in shade. Figure 2 is a reproduction of a typical SEM image of a section of sheep skin which has been treated as described above.

Using SEM, the method is particularly suited to the determination of wool fibre and follicle characteristics at the level of detail desirable when such characteristics are to be a basis for selecting sheep for fine wool production.

Accordingly, a further aspect of this invention is a method of selecting sheep for fine wool production which comprises the steps of forming a detailed impression of the skin surface of an animal and treating that impression so that on examination by SEM, each fibre or bundle of fibres may be identified as a substantially circular hole within an annulus distinguishable by shade from the hole and regions surrounding the annulus.

Without limiting the scope of the invention, the following is an outline of practicable procedures for obtaining an impression suitable for SEM examination:

After clipping and washing, the midside region of the selected animal is closely shaved using, say, a blade razor and wetting agent; the shaved surface is then cleansed with water and dryed before applying an impression-forming material. The formation of a detailed and durable impression will be enhanced if the resin is worked gently into the skin surface, followed by the addition of further resin for strengthening.

For the greatest detail it is preferable for the resin to be in a fairly fluid condition at the time of application, and in the case of vinyl polysiloxane resins this is readily achieved by heating the components prior to mixing - a procedure which also speeds up setting. The formation of a detailed and conveniently shaped impression may further be facilitated by pressing the resin onto the skin surface from a heated metal mould; the heat from the mould acting to accelerate setting, and the mould rigidity tending to promote uniformity of pressure over the area where the resin is in contact with the skin surface.

To minimise wrinkling, the skin may be gently held taut just prior to applying the resin.

After polymerisation, the impression may be removed, and made electrically conductive in order to facilitate SEM examination. Suitable procedures for rendering an impression electrically conductive include rotary shadowing or sputter coating with a metal or mixture of metals selected from, for example, gold, silver, palladium, platinum or aluminium

It is believed that the method described herein offers the basis of a practical and economic measuring service for wool breeders, whereby skin surface impressions prepared on-farm by breeders would be interpreted by e.g. agencies having the appropriate facilities and expertise. Thus, a further feature of this invention is a kit comprising materials suitable for the preparation of skin surface impressions, for example the kit may contain means for cropping a prescribed area of skin, an impression-forming resin, means for applying or detaching the resin impression from a prescribed area of skin, and instructions for using such components.

Confirmation of the interpretation of the skin surface impressions is afforded by a refinement of this invention. An impression may be seen as a "negative replica" of the skin surface; in the refinement, the "positive replica", i.e. a model of the skin surface, is formed by applying to a skin impression a material able to conform to the contours of the impression, and which after removal can be inspected by any

suitable means, such as SEM. We have found Araldite epoxy resin (Selleys Chemical Company Pty Ltd) to be an effective material for this purpose.

While the "positive replica" may not be readily amenable to automated image analysis, and therefore not made use of in the course of routine measurement, it is likely to be of value as a research tool and in the development of image analysis software.

It is to be appreciated that this invention is not restricted to examination of fibre populations in wool growing animals, but is likely to find application where such examinations are of interest in respect of other fibre producing animals.

CLAIMS

1. A method of characterising fibre follicle populations in an animal which comprises the steps of:

(a) cropping a selected area of the skin of the candidate animal;

(b) applying to the cropped area a settable material adapted to conform closely to and thereby form a detailed impression of the skin surface;

(c) examining the surface of the so-formed skin impression to determine the incidence of solitary fibres, bundles of fibres or the number of fibres per bundle.

2. A method according to any previous claim wherein examination of the skin impression is carried out by scanning electron microscopy.

3. A method according to any previous claim wherein the settable material is vinyl polysiloxane.

4. A method according to any previous claim wherein the skin impression is made electrically conductive so that on inspection by a scanning electron microscope, the location of a fibre(s) is observed as a dark, more or less circular hole surrounded by an annulus which is light in shade relative to surrounding regions of the skin.

5. A method according to claim 4 wherein the skin impression is rendered electrically conductive by rotary shadowing or sputter coating with a metal or metals selected from gold, silver, palladium, platinum or aluminium.

6. A method according to any previous claim wherein the incidence of fibres, fibre bundles and/or number of fibres per bundle is determined by applying computerised image analysis techniques to an electron microscope scan of the skin impression.

7. A method of selecting sheep for fine wool production which comprises determining the characteristics of an animal's fibre population and associated follicle population by the method of any previous claim.

8. A kit for use in performance of a method according to any previous claim, said kit comprising a material, or agents for producing such material, capable of forming a detailed, durable impression of the skin surface when applied to the skin of a candidate animal.

Dated this 1st day of May 1995

COMMONWEALTH SCIENTIFIC AND INDUSIRIAL RESEARCH ORGANISATION

ABSTRACT

A method of characterising fibre follicle populations in an animal is disclosed which comprises the steps of cropping fibre from a selected area of the skin of the animal and applying to the selected area a suitable material such as vinyl, polysiloxane resins which are adapted to conform closely to and thereby form a detailed impression of the skin surface. The resins may be heated to place them in a fluid condition at the time of application. After setting the set resin is removed to provide a formed impression and the formed impression is examined to determine the incidence of solitary fibres, bundles of fibres and the number of fibres per bundle.

17793/95

1/2

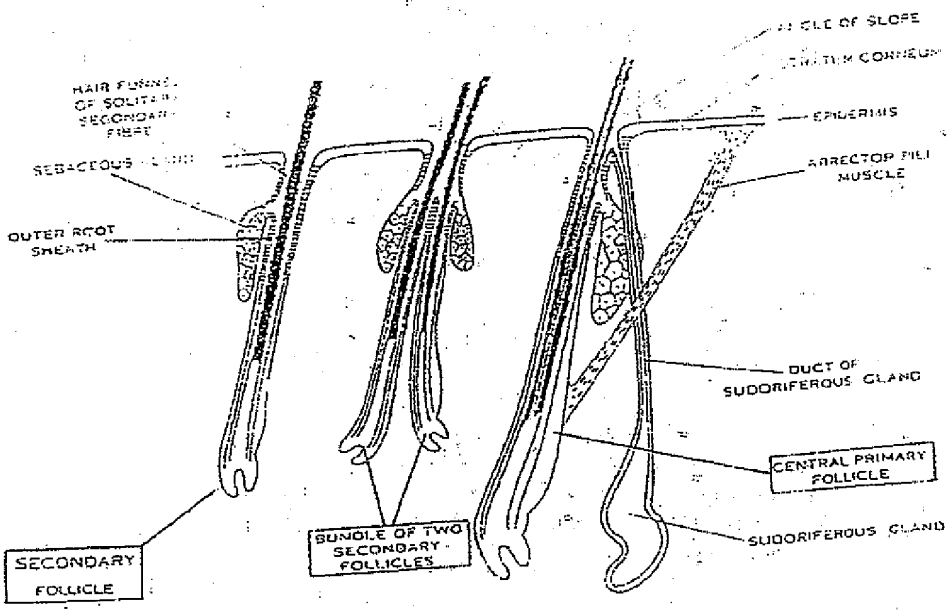

FIGURE 1

2/2

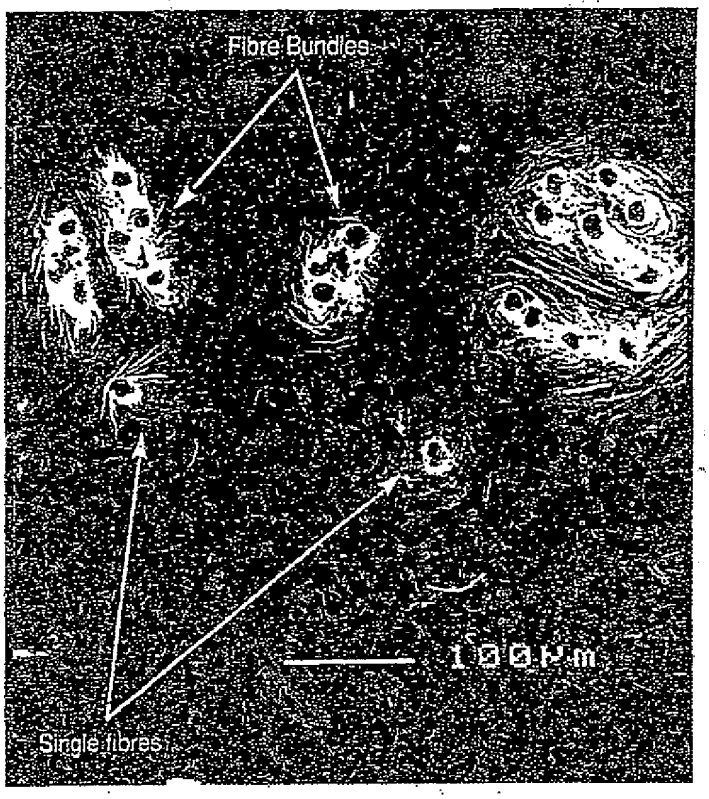

Figure 2